Paternal age is associated with mitochondrial vulnerability to sperm cryopreservation

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Study question (25 words): Is paternal age associated with lower mitochondrial activity in fresh and cryopreserved semen?

Summary answer (25 words): Paternal age does not appear to severely compromise sperm mitochondrial activity in fresh semen but determines higher mitochondrial vulnerability to sperm cryopreservation.

What is known already (100 words): The impact of paternal age on male fertility has not been fully clarified although growing evidence suggests age-related loss of sperm quality. Mitochondrial activity has been recognized as a reliable marker of sperm functionality, reflecting motility, vitality and fertilization competence. Sperm cryopreservation has been largely utilized in fertility preservation and ART schemes, despite its potential harm on cell membranes including those of the mitochondria. The influence of paternal age on sperm vulnerability to cryopreservation-induced cell damage is still not known.

Study design, size, duration (75 words)

Twenty three normospermic patients, 11 of which ≤ 35 (non-APA) and 12 ≥ 42 (APA), provided semen samples by masturbation after 1-5 days of abstinence, between April and August of 2022. A 250 µl semen aliquot was frozen. Sperm concentration, motility, vitality, morphology and mitochondrial functionality were compared in fresh semen from non-APA vs. APA patients, while motility and mitochondrial functionality were compared in thawed semen from the same groups with a two-tailed Student t-test.

Participants/materials, settings, methods (75 words)

Fresh and thawed semen samples were provided by patients under evaluation for couple infertility treatment in our fertility center. Semen was cryopreserved with CryoSperm (Origio) and analyzed after rapid thawing and washing/dilution in Sperm Preparation Medium (Origio). Sperm vitality was assessed with VitalSrceen (FertiPro) and mitochondrial functionality was evaluated with MitoTracker Red CMXRos (Cell Signalling Technology), through the percentage of stained cells and fluorescence intensity measured in 75 spermatozoa per patient with the ImageJ software.

Main results and the role of chance (200 words)

Mean ages for non-APA and APA patients were 32.6 and 45.3 years, respectively. Age groups did not differ for any of the parameters assessed in fresh semen (volume: 2.3±1.1 vs. 2.6±1.2 mL; concentration: 58±32 vs. 47±20 x10ˆ6/mL; rapid progressive motility 5±5 vs. 5±8%; slow progressive motility 40±10 vs. 36±10%; non-progressive motility 10±5 vs. 10±3%; immotile: 44±9 vs. 49±13%; normal morphology: 6±3 vs. 5±1%; vitality: 71±8 vs. 66±15%, for non-APA and APA, respectively). However, non-APA patients presented a higher percentage of spermatozoa with rapid progressive motility (5±7 vs. 0%; p=0.04) and a lower percentage of immotile spermatozoa (76±12 vs. 88±9; p=0.02) after thawing. Regarding mitochondrial functionality, no differences were observed in fresh semen from different age groups [93±11 vs. 92±7% stained cells; 3.33±1.57 vs.

3.35±1.49 (fluorescence arbitrary units), for non-APA and APA, respectively]. In cryopreserved semen, however, although age groups did not differ for the percentage of stained spermatozoa (92±10 vs. 90±5%), fluorescence intensity was higher in spermatozoa from non-APA patients (2.53±1.63 vs. 1.76±0.54; $p<0.01$).

Limitations, reasons for caution (50 words)

Our study is limited by the potential interference of confounding factors not equally distributed in age groups. The conclusions from this study must be confirmed in other patient populations with different race/genetics and habits.

Wider implication of the findings (50 words).

Our study sheds light on the impact of paternal age on sperm quality, a topic highly relevant to reproductive medicine still not fully understood. We provide evidence that APA is associated with higher vulnerability of mitochondria to the stress induced by cryopreservation and its metabolic consequences.

Study funding/competing interests

All authors declare no conflict of interest.

Trial registration number

NA